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Colony Formation and Inversion in the Green Alga *Eudorina elegans*

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With 27 Figures

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Summary

During development of daughter coenobia in the volvocalean alga *Eudorina* a rapid synchronized series of mitotic divisions and cytokineses gives rise to a slightly cup-shaped, patterned array of 16 or 32 cells, the plakea; the nuclei and centrioles of each cell lying at the concave face and the plastids at the convex face. Each cell is connected to its neighbours by cytoplasmic bridges. All cells within a plakea simultaneously elongate and enlarge their nuclear poles; while remaining interconnected by the cytoplasmic bridges at their plastid poles. The result is inversion of the developing coenobia so that the nuclei and centrioles come to lie on the convex, outer surface. Inversion is inhibited by colchicine and cytochalasin B. Both lengthening of the cells and expansion of their nuclear end is apparently mediated by microtubules. Striations on the plasmalemma encircling the bridges are thought to stablize the membrane at these sites during inversion.

1. Introduction

The freshwater colonial volvocalean alga *Eudorina* contains 16–64 cells arranged as a hollow sphere in a glycoprotein matrix. Apparently all members of the *Volvocales* having spherical colonies undergo inversion, where, during their development, the colony turns itself inside out so that the flagella are extended from the exterior, convex face of the hollow ball of cells. While there are detailed light microscope descriptions of development in many of the colonial *Volvocales*, *e.g.*, *Gonium* (HARPER 1912, HARTMANN 1924), *Pandorina* (MORSE 1943, TAFT 1941) *Eudorina* (CONRAD 1913, HARTMANN 1924, GOLDSTEIN 1964) and *Volvox* (POCOCK 1933) I am not aware of any reports of ultrastructural studies on coenobial differentiation in these algae other than PICKETT-HEAPS' (1970) on aspects of inversion in *Volvox*. The only published fine structural details on *Eudorina* is HOBBS' (1971) description of some aspects of vegetative cells. Here I report on colony formation in *E. elegans* paying particular attention to the mechanism of inversion.

2. Materials and Methods

Eudorina elegans Ehrenberg, strain 24/1a, from the Culture Centre of Algae and Protozoa, Cambridge, England, was grown in POCOCK's (1960) Medium at 20 °C on a 15/9 hours light/dark cycle. Coenobia in which daughter colonies were developing were fixed at room temperature for 1 hour in 1% glutaraldehyde made up in growth medium. The coenobia were washed with growth medium and subsequently post fixed for 1 hour at room temperature with 1% OsO_4, again made up in growth medium. Another fixation schedule where coenobia were fixed in 1% glutaraldehyde for only 10–20 seconds at room temperature before 4% OsO_4 was added directly to the cells in glutaraldehyde so that the final concentration of OsO_4 was 1% gave excellent preservation. This latter fixation was terminated after 10–20 minutes when the mixed fixative solution began to turn brown. Fixed coenobia were washed in distilled water and dehydrated at 0 °C with acetone and embedded in SPURR's (1969) epoxy resin. Thin sections of selected coenobia were stained with uranyl acetate and lead citrate and examined in an Hitachi H 500 electron microscope.

Colony formation was recorded cinematographically using Bolex-Wild time-lapse equipment on a Zeiss Universal microscope.

Parental coenobia containing daughter coenobia at various developmental stages were treated with 0.4% colchicine (Sigma Chemical Co.) freshly made up in growth medium. These coenobia were examined at various times with an inverted microscope to ascertain the effect of this drug on colony development. Cytochalasin B (Aldrich Chemical Co.) was dissolved in dimethyl sulphoxide (DMSO) and diluted to 10–50 µg/ml with growth medium so that the concentration of DMSO was always less than 0.5%. Parental coenobia containing developing daughter coenobia were treated with cytochalasin B for periods up to 24 hours. Some coenobia treated with colchicine or cytochalasin B were processed for electron microscopy as described above, others were filmed by time-lapse cinematography.

3. Results

Under my culture conditions, coenobia of *Eudorina elegans* normally contain 16 or 32 cells and generally all of these cells are gonidia (Fig. 1), that is, cells which will eventually produce daughter colonies. Some colonies con-

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Abbreviations used in Figures: *b* = cytoplasmic bridge, *c* = centriole, *ch* = chromosomes, *cl* = chloroplast, *cv* = contractile vacuole, *er* = endoplasmic reticulum, *f* = cleavage furrow, *fl* = flagellum, *g* = golgi body, *n* = nucleus, *ne* = nuclear envelope, *t* = microtubules

Fig. 1. Mature 32 cell coenobium of *Eudorina;* arrows indicate flagella. Phase contrast ×180

Fig. 2. Mature 32 cell coenobium of *Eudorina*, the 4 somatic cells (arrows) will not produce daughter coenobia. Phase contrast ×180

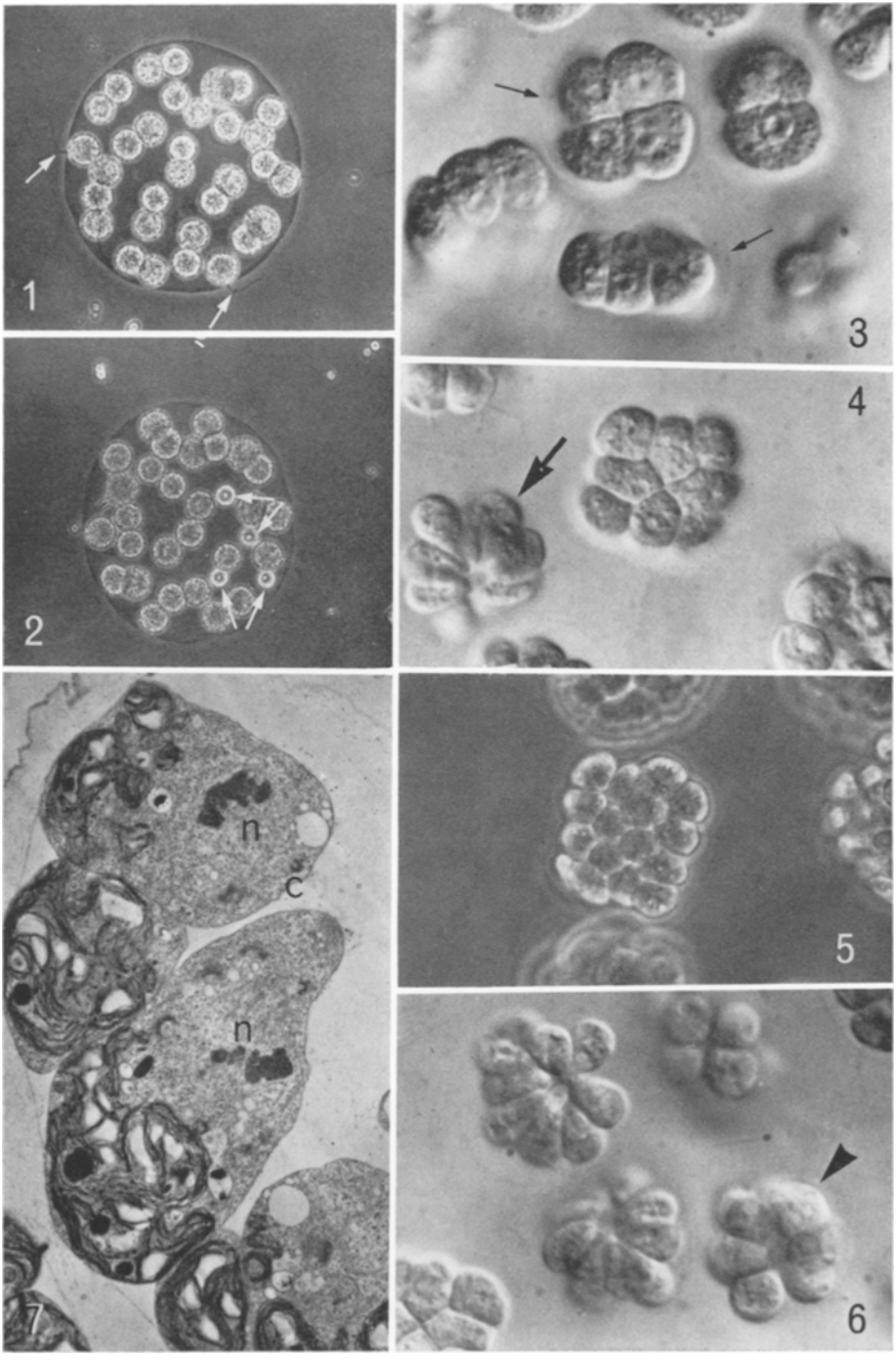
Fig. 3. Part of a parental coenobium in which daughter colony development is taking place; two, four and eight cell stages are evident. Note the vesicle surrounding each developing colony (arrows). Nomarski optics ×1,000

Fig. 4. An eight cell stage in colony development showing the characteristic pattern of cells. The other daughter coenobium (arrow) is inverting. Nomarksi optics ×1,100

Fig. 5. Sixteen cell plakea. Phase contrast ×800

Fig. 6. Inverting daughter coenobia and one that has recently inverted (arrowhead). Note the elongate conical shape of the inverting cells and that the cells in the coenobium that has inverted are shorter but still closely appressed (*cf.*, Fig. 25). Nomarski optics ×1,000

Fig. 7. Electron micrograph of part of a developing coenobium the cells of which are undergoing mitosis simultaneously. ×6,000



Figs. 1–7

tained 2 or 4 small somatic cells (Fig. 2) which did not reproduce. A rapid series of nuclear and cell divisions gives rise to a 16 cell plakea (Figs. 4–6). Mitotic nuclei were infrequently seen and as a result I made no attempt to study mitosis in detail. My limited observations revealed that mitosis is highly synchronous within a developing coenobium (Fig. 3) and that the nuclear envelope remains essentially intact except for polar fenestrae. Kinetochores are not well developed and centrioles lie at least at one of the poles of the spindle (Fig. 8).

Cytoplasmic cleavages are predicted by phycoplast microtubules (Fig. 9); furrowing commences near the nuclei and progresses towards the plastids (Figs. 9 and 10). Most often, at least at the 2–8 cell stage, furrowing is incomplete, the cells remaining joined by broad cytoplasmic interconnections (Figs. 10 and 11). Cytoplasmic bridges, which also link the cells (Figs. 12 and 13) have a diameter of 140–250 nm, mean 196 nm. While these bridges can occur anywhere along the margins of dividing cells (Figs. 13 and 16), they are more commonly observed adjacent to the plastid (Figs. 21–24). It is not unusual to find cytoplasmic bridges traversed by endoplasmic reticulum (Fig. 12) and microtubules (Figs. 14 and 17). The precisely oriented cytokineses produce a characteristically patterned arragement of cells (Figs. 4–6) which at the 16 cell stage is slightly cup shaped with the nuclei and centrioles lying on the concave face (Fig. 11).

All cells in the developing colony lengthen and undergo a profound change in shape from being conical with the nucleus lying at the apex of the cone, to cylindrical and then conical again but with the plastid lying at the apex and the nucleus at the base (*c.f.*, Figs. 11 and 25). While the cells are changing shape they all remain interconnected by bridges. As a result of the change in shape of each cell while still remaining joined to those surrounding it, the nuclear end of the cells come to lie at the convex face of the colony.

Microtubules radiate from dense material adjacent to the basal bodies (Fig. 15) from the time the cells elongate to when they round up following inversion. The tubules lie close to the plasmalemma and run from near the basal bodies to the end of the cell occupied by the plastid (Figs. 14, 16, and 21). Other tubules lie at right angles to these longitudinal ones at the nuclear end of the cell (Fig. 20). Treatment of 16 or 32 cell plakeas with 0.4% colchicine eliminates most of the microtubules and inhibits inversion. Colchicine treated cells do not elongate significantly nor does their nuclear end enlarge. Washing out the colchicine after 1 hour allows inversion to proceed apparently normally.

The plasmalemma adjacent to bridges interconnecting elongate cells appears striated (Figs. 17–19), such decoration not being apparent in cells that are not elongate. Also, the plasmalemma of the bridges appears to be underlain with densely staining material (Figs. 17, 18, and 23). Treatment of 16 cell plakeas with cytochalasin B at concentrations varying from 25–50 μg/ml for

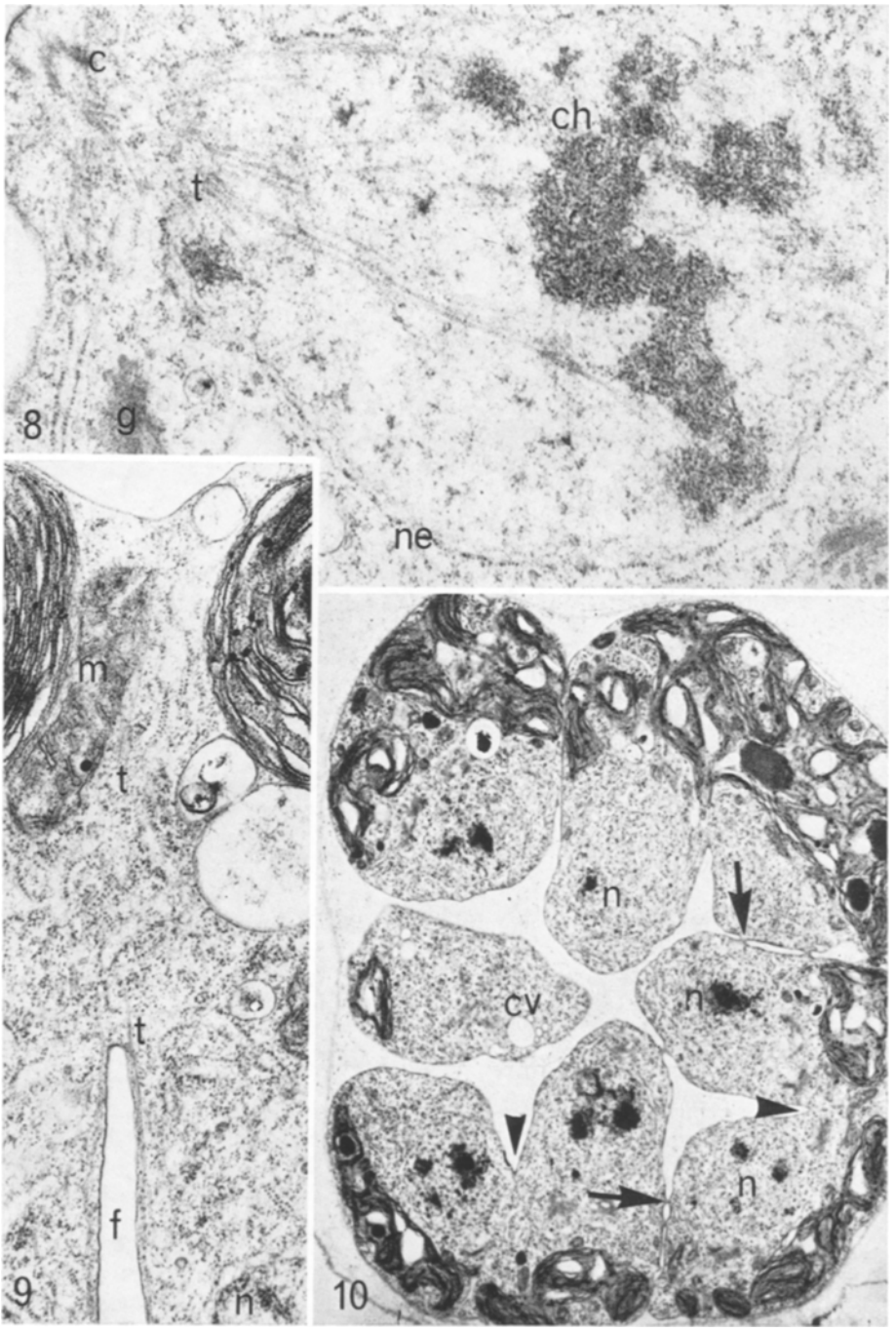


Fig. 8. Mitotic nucleus showing a centriole at the polar fenestra, the otherwise intact nuclear envelope and diffuse kinetochores. ×28,000

Fig. 9. Developing cleavage furrow and associated microtubules. ×28,000

Fig. 10. Eight cell developing colony showing the perpheral localization of the plastids and the central position of the nuclei. Bridges (arrows) and incomplete furrows (arrowheads) join the cells. ×5,000

2–5 hours prevents inversion, but flagellar development apparently proceeds normally. Transfer of colonies that had been treated with cytochalasin B for up to 3 hours to normal growth medium permits inversion to be completed, however the resulting colonies are frequently abnormal.

Flagella appear during inversion (Fig. 26) but remain short and relatively inactive until inversion is complete. Following inversion, the cells apparently adhere laterally (Fig. 24), their interconnecting bridges break and the cells become rounded (Fig. 25) and lengthen their flagella which protrude through a fluted channel in the new coenobial sheath (Fig. 27). Gelatinization of the parental coenobial sheath releases daughter colonies. I have never observed bridges or any other cytoplasmic interconnection between mature cells. During cell division of the gonidia the parental flagella continue to beat, although not as strongly or as well coordinated as when the cells are not dividing. I have not been able to ascertain by electron microscopy how these parental coenobial flagella are attached.

4. Discussion

The rapid sequence of karyokinesis and cytokinesis in *Eudorina* have to be precisely oriented in order to give rise to the intricately patterned arrangement of uninucleate cells in the 16 or 32 cell plakea which subsequently inverts. Mitosis has been described in detail in *Chlamydomonas* (JOHNSTON and PORTER 1968, TREIMER and BROWN 1974, COSS 1975), *Tetraspora* (PICKETT-HEAPS 1973) and *Volvox* (DEASON and DARDEN 1971). My observations on mitosis in *Eudorina* did not reveal any significant variation to that found in these other volvocalean algae.

Apparently all the colonial *Volvocales*, irrespective of the number of cells they ultimately contain, undergo the same initial sequential pattern of divisions. Unlike *Gonium* which remains a planar, typically 16 cell colony in which all cells divide to produce daughter colonies, the spherical colonies of

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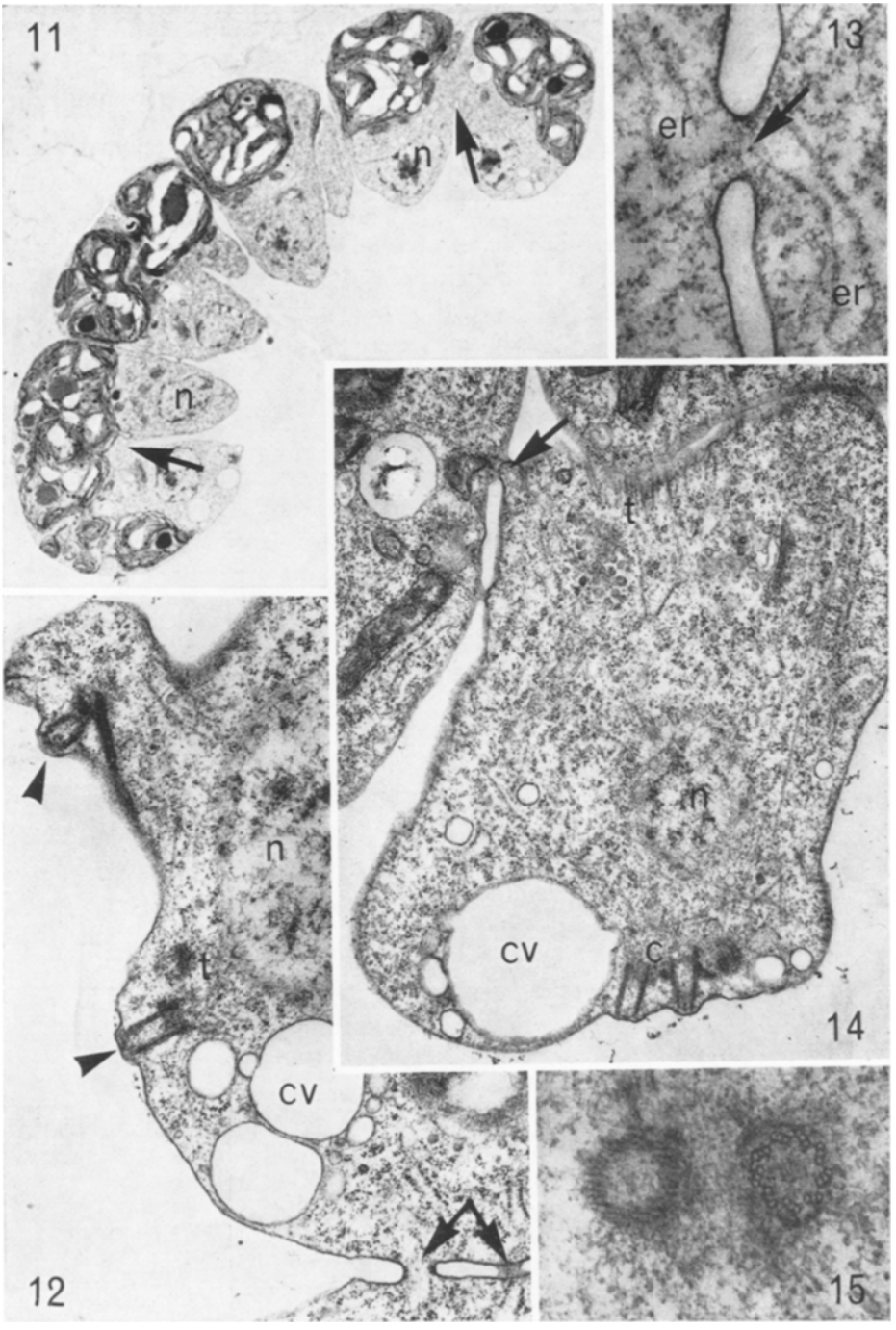
Fig. 11. Section through a 16 or 32 cell plakea in which some cells are still joined by broad cytoplasmic interconnections (arrows). Note the position of the nuclei on the concave face (*cf.*, Fig. 25). ×4,000

Fig. 12. Basal region of a cell which is part of an 8 cell developing coenobium. The cell is joined to another by bridges (arrows). The centrioles (arrowheads) of this cell have divided; their separation and associated microtubules suggest that this cell is approaching mitosis. ×23,000

Fig. 13. Cytoplasmic bridge traversed by endoplasmic reticulum (arrow). ×49,000

Fig. 14. Oblique section through a cell in a colony at an early stage of inversion. Microtubules apparently radiate from near the centrioles. Note the concentration of tubules at the opposite side of the cell and the tubule which may traverse the cytoplasmic bridge (arrow). ×20,000

Fig. 15. A pair of centrioles surrounded by dense material from which the microtubules apparently emanate. ×64,000



Figs. 11–15

the "higher" *Volvocales* contain both small somatic cells and gonidia (larger cells which give rise to daughter colonies). *Eudorina* is variable, some species regularly containing both somatic and generative cells while others, such as the species studied here, *E. elegans,* may or may not show somatic-generative cell differentiation (GOLDSTEIN 1964). Amongst a number of hypotheses that have been advanced to account for the non-random distribution of somatic and generative cells in coenobia, the one best supported by experiments is that there is an early determination of areas of the cytoplasm of the parental cell into zones that will eventually become differentiated (GERISCH 1959). GOLDSTEIN (1967) further developed this idea suggesting that a cytoplasmic component, an organelle or substance, would fail to segregate randomly on division and remain peripherally located during the cell divisions that give rise to the plakea. My observations however do not reveal any obvious segregation of organelles, and the existence of broad cytoplasmic bridges which interconnect dividing and inverting cells would presumably permit equilibration of any substance that was not bound to, or contained by, cellular components that could not traverse the bridges. How then "non-equivalence" (LEWIS and WOLPERT 1976) between differentiating somatic and generative cells is developed and maintained while the bridges are intact remains enigmatic.

The development of cytoplasmic bridges in *Volvox* results from incomplete cytokinesis (BISALPUTRA and STEIN 1966, PICKETT-HEAPS 1970), and the bridges between cells of *Eudorina* arise similarly. In *Eudorina* their number and distribution appears to be random during cytokinesis of the parental cell, but only a few remain between adjacent cells at the time of inversion, these being congregated at the extreme chloroplast end of the cells. Whether or not these bridges persist between mature cells varies amongst species of *Volvox* (MARCHANT 1976 a). Bridges have been reported to interconnect cells of *Gonium* and *Pandorina* but the occurrence of these intercellular connections

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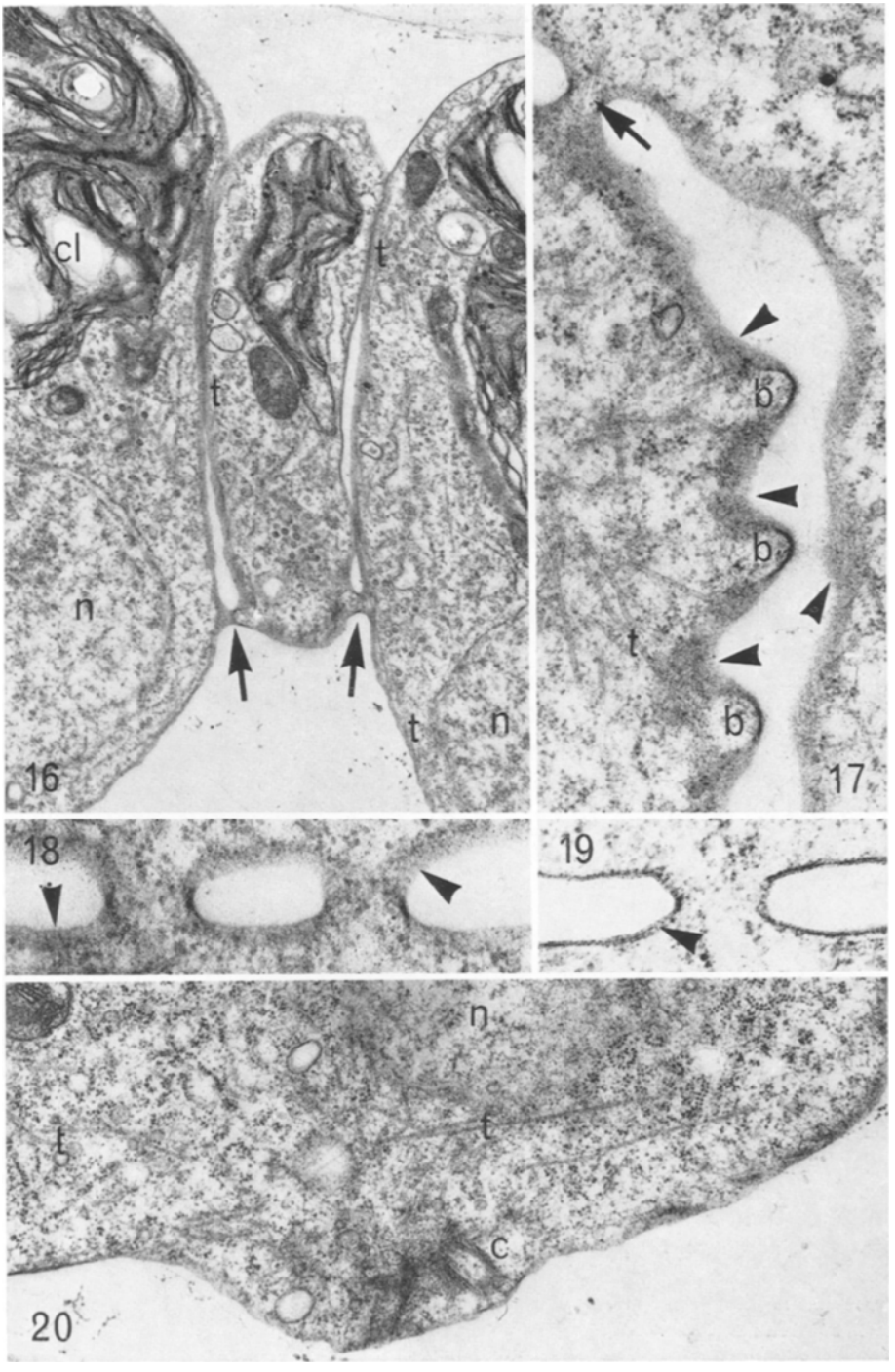
Fig. 16. Three cells of a plakea fixed shortly before inversion. Note the peripheral microtubules (*t*) and two bridges (arrows) interconnecting the cells at their nuclear ends. The bridge on the left appears stretched and both bridges would have broken for inversion to be completed successfully. ×13,000

Fig. 17. Margin of cells in an inverting coenobium. The membrane adjacent to the bridges (*b*) shows striations (arrowheads) which apparently encircle the bridges with their densely stained membrane. Microtubules traverse one of the bridges (arrow). ×42,000

Fig. 18. Striations on the plasmalemma around two adjacent intercellular bridges. ×67,000

Fig. 19. Longitudinal section of an intercellular bridge showing dense material on the cytoplasmic face of the membrane with the regularly spaced striations embedded in it (arrowhead). ×62,000

Fig. 20. Nuclear end of a cell in an inverting coenobium. Microtubules apparently emanate from the region of the centrioles, some lie approximately parallel with the membrane adjacent to the centrioles. ×36,000



Figs. 16–20

has not been confirmed ultrastructurally (STEIN 1965). In *Eudorina* they break down shortly after inversion.

The function of bridges between cells of the various members of the *Volvocales* has been the subject of considerable speculation. A popular but untested view is that the bridges of *Volvox* are involved in the coordination of flagellar beat (DOLZMANN and DOLZMANN 1964). Such a function is unlikely as mature coenobia of some species of *Volvox*, lacking bridges, are able to swim normally (IKUSHIMA and MARUYAMA 1968). The diameter of bridges of *Eudorina* (and also *Volvox*) would prevent the intercellular movement of large organelles but they are large enough to accommodate elements of endoplasmic reticulum and microtubules and do not appear to contain any structure that would impede the movement of ions or freee molecules. Hence, they almost certainly function as channels of intercellular communication and are thus analogous in function, but clearly not in structure, to the plasmodesmata of some other algae and the higher plants (MARCHANT 1976 a). There are at least two developmental events during colony formation in which the activities of daughter cells must be coordinated. One is maintaining synchrony of mitotic divisions and cytokineses to produce the characteristic patterned arrangement of cells within the plakea, the other is integrating the events of inversion.

Inversion is a complex process involving the simultaneous lengthening of all the cells in the plakea, broadening of their nuclear end and constriction of the plastid-containing end of each cell where the cells are interconnected by bridges. The lengthening of the cells and the broadening of their nuclear end are apparently brought about by microtubules as treatment of plakeas with colchicine, a drug that interferes with microtubule polymerization, inhibits the change in shape of the cells and blocks inversion. Very few microtubules are present in colchicine treated cells. Whether the lengthening and broadening of the cells is achieved by the same array of microtubules or whether there are two independent systems has not been resolved. It appears though that microtubules involved in both the lengthening and broadening arise from a diffuse region around the centrioles. A similar involvement of microtubules in lengthening cells in inverting colonies of *Volvox* has been demonstrated by RENNER and PICKETT-HEAPS (PICKETT-HEAPS 1975). In

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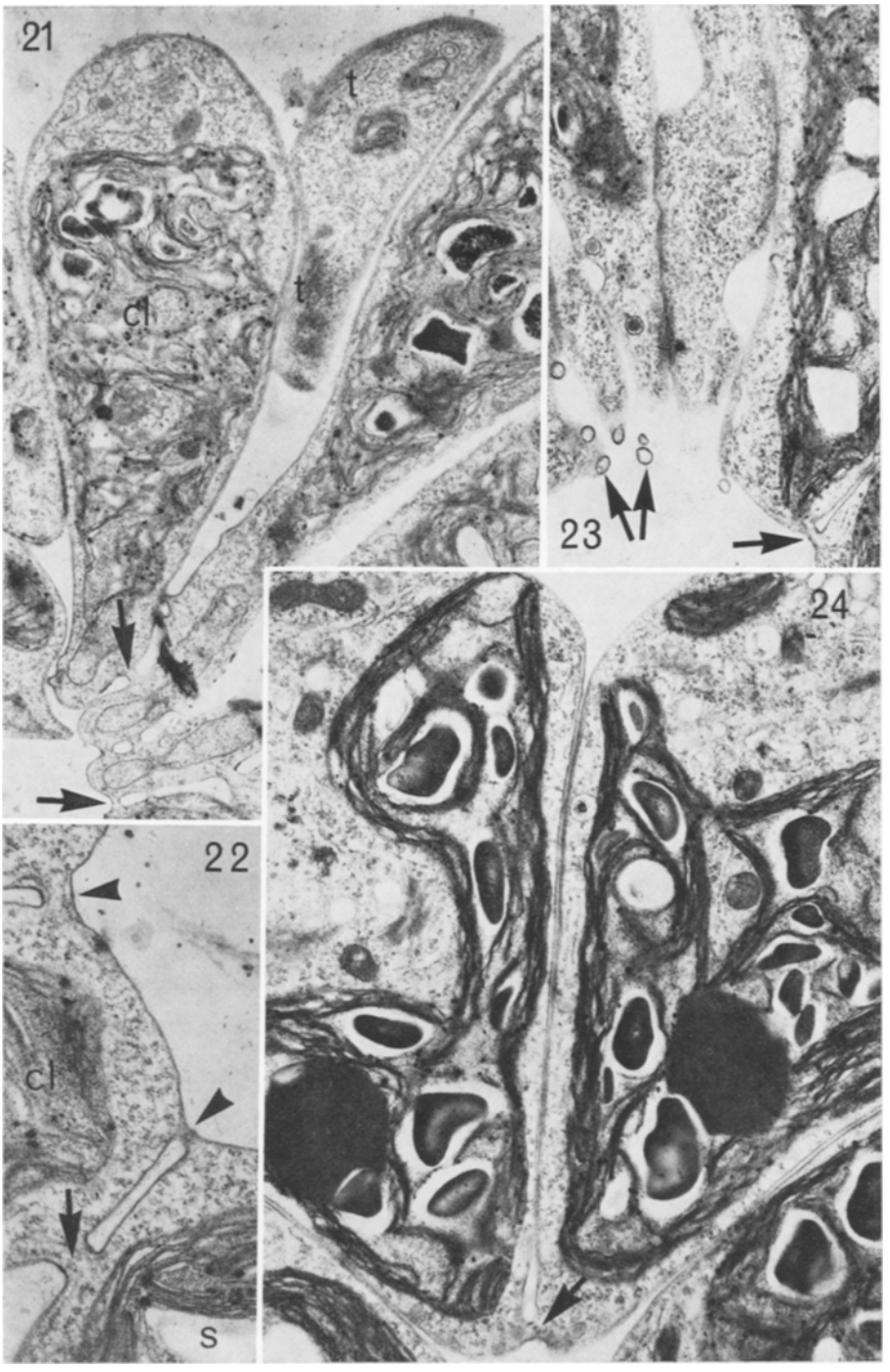
Fig. 21. Cells of a coenobium which is undergoing inversion showing the bridges between cells, the perpheral microtubules and the basal constriction. ×11,000

Fig. 22. Bridges (arrowheads) interconnecting cells of a coenobium which had recently completed inversion. The chloroplast ends of the cells are enlarging and the bridges, with a conspicuous exception (arrow), are located at the "corners" of the cells. ×26,000

Fig. 23. Plastid ends of cells in a coenobium having recently inverted. The bridges, with their densely staining membranes (arrows), are breaking down. ×13,000

Fig. 24. Cells at a late stage of inversion of the coenobium closely appressed laterally and interconnected by bridges (arrow). ×14,000



Figs. 21–24

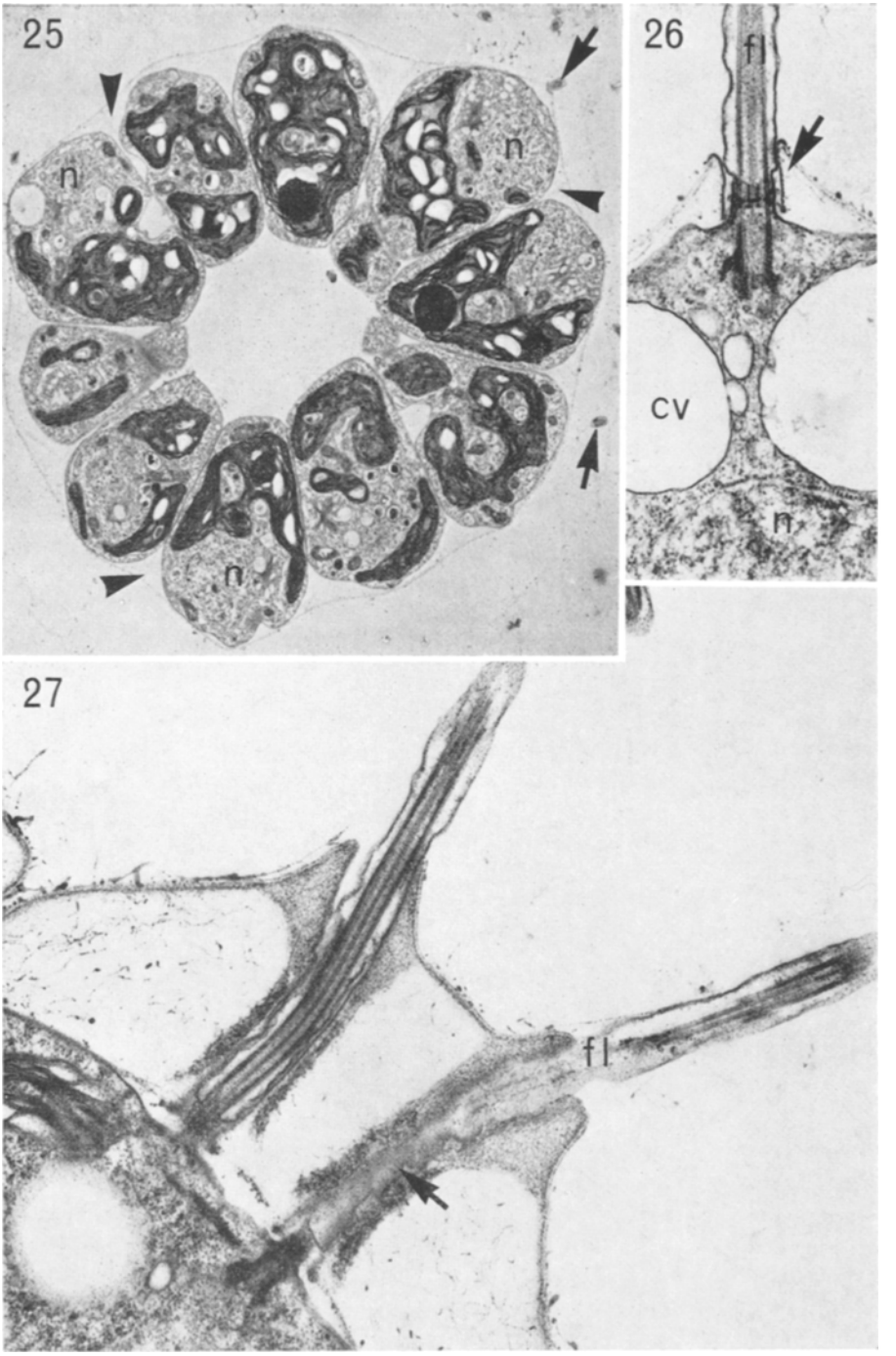


Fig. 25. Recently inverted colony. Partitions in the sheath between the cells (arrowheads) are developing and the cells are losing their conical shape, becoming rounded as well as extending their flagella (arrows). ×4,500

Fig. 26. Recently extended flagellum arising from a basal body flanked by contractile vacuoles. Development of the outer layer of the sheath and its differentiation around the flagellum (arrow) is underway. ×27,000

Fig. 27. A pair of flagella extended from a mature cell. Note the various layers of the sheath and the fluted channel through the sheath occupied by the flagella (arrow). ×20,000

Volvox however there has been no report of microtubules involved in the broadening of the nuclear end.

Microtubules are clearly involved in the change of cell shape during gastrulation in birds (GRANHOLM and BAKER 1970) and echinoderms (GIBBINS *et al.* 1969) and urodele neurulation (BURNSIDE 1971). While the wall of most plant cells determines their shape those cells lacking a wall *e.g.*, the alga *Ochromonas* (BOUCK and BROWN 1973) rely on microtubules for the maintenance of their distinctive cell shape. Similarly, microtubules can influence the development of the complex shape of some plant cells before their acquisition of a wall *e.g.*, *Pediastrum* and *Sorastrum* (MARCHANT 1974 a, b),. And as in animal cells (TILNEY and GIBBINS 1969) the development of cell form is disrupted by drugs (*e.g.*, colchicine and IPC) which interfere with microtubule polymerization (BROWN and BOUCK 1974, MARCHANT and PICKETT-HEAPS 1974) highlighting the widespread microtubular function in determining cell shape.

PICKETT-HEAPS (1970) reported spiral or concentric striations or microfilaments on the plasmalemma of cytoplasmic bridges between inverting cells of *Volvox*. Similar striations are also obvious in inverting colonies of *Eudorina*. Striations similar to those in *Eudorina* appear on the plasmalemma of *Sorastrum* at a site that becomes constricted (MARCHANT 1974 b). In yeast cells, BYERS and GOETSCH (1976) found striations, which may be filaments, closely associated with the plasmalemma at the neck of developing buds; the neck did not contract and the filaments disappeared as cytokinesis began. BYERS and GOETSCH suggest that the filaments may limit the size of the neck between the parental cell and developing bud or else may be involved in controlling the deposition of components of the cell wall. I consider it unlikely that the striations in *Eudorina*, are contractile and that similar to the decoration of the plasmalemma of yeast they may be involved in providing a constraint on the size of the cytoplasmic bridges and stabilizing the membrane at these sites where, during inversion, the cells hinge, pushing against one another.

Inversion of daughter coenobia is blocked by cytochalasin B, the mode of action of which is unclear as HEPLER and PALEVITZ (1974) rightly point out, interpretations based on this drug's effects must be regarded with caution. One possible action of cytochalasin is the disruption of actin but no ultrastructural alteration of the microfilament bundles is apparent in internodes of *Chara* and *Nitella* following arrest of streaming in these cells with this drug (BRADLEY 1973). Likewise, there was no disruption of the striations surrounding the bridges in inverting coenobia of *Eudorina*. Actin has been detected by heavy meromyosin binding in other algae (WILLIAMSON 1974, PALEVITZ and HEPLER 1975, MARCHANT 1976 b) and it is reasonable to propose that some of the change in cell shape occurring during inversion are mediated by actin, the role of the microtubules being rather that of a cytoskeleton as distinct from subcellular "muscle".

Acknowledgements

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